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NEWS 13	SEP 17	CAplus coverage extended to include traditional medicine patents
NEWS 14	SEP 24	EMBASE, EMBAL, and LEMBASE reloaded with enhancements
NEWS 15	OCT 02	CA/CAplus enhanced with pre-1907 records from Chemisches Zentralblatt
NEWS 16	OCT 19	BEILSTEIN updated with new compounds
NEWS 17	NOV 15	Derwent Indian patent publication number format enhanced
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NEWS 19	NOV 30	ICSD reloaded with enhancements
NEWS 20	DEC 04	LINPADOCDB now available on STN
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NEWS 22	DEC 17	USPATOLD added to additional database clusters
NEWS 23	DEC 17	IMSDRUGCONF removed from database clusters and STN
NEWS 24	DEC 17	DGENE now includes more than 10 million sequences
NEWS 25	DEC 17	TOXCENTER enhanced with 2008 MeSH vocabulary in MEDLINE segment
NEWS 26	DEC 17	MEDLINE and LMEDLINE updated with 2008 MeSH vocabulary
NEWS 27	DEC 17	CA/CAplus enhanced with new custom IPC display formats
NEWS 28	DEC 17	STN Viewer enhanced with full-text patent content from USPATOLD
NEWS 29	JAN 02	STN pricing information for 2008 now available
NEWS 30	JAN 16	CAS patent coverage enhanced to include exemplified prophetic substances
NEWS 31	JAN 28	USPATFULL, USPAT2, and USPATOLD enhanced with new custom IPC display formats
NEWS 32	JAN 28	MARPAT searching enhanced
NEWS 33	JAN 28	USGENE timeliness enhanced
NEWS 34	JAN 28	TOXCENTER enhanced with reloaded MEDLINE segment
NEWS 35	JAN 28	MEDLINE and LMEDLINE reloaded with enhancements

NEWS EXPRESS 19 SEPTEMBER 2007. CURRENT WINDOWS VERSION IS V5.2.

CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),  
AND CURRENT DISCOVER FILE IS DATED 19 SEPTEMBER 2007.

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FILE 'LIFESCI' ENTERED AT 12:12:17 ON 29 JAN 2008  
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=> s phosphatidylserine(w)bind?  
L1 628 PHOSPHATIDYL SERINE(W) BIND?  
  
=> s l1 and annexin?  
L2 162 L1 AND ANNEXIN?  
  
=> dup rem l2  
PROCESSING COMPLETED FOR L2  
L3 77 DUP REM L2 (85 DUPLICATES REMOVED)  
  
=> s "annexin V" and l3

L4

53 "ANNEXIN V" AND L3

=> d 40-53 ibib ab

L4 ANSWER 40 OF 53 HCPLUS COPYRIGHT 2008 ACS on STN  
ACCESSION NUMBER: 2005:316302 HCPLUS  
DOCUMENT NUMBER: 142:390959  
TITLE: Identification, assessment, prevention, and therapy of rheumatoid arthritis  
INVENTOR(S): Guild, Braydon C.; Liao, Hua; Jones, Michael D.; Wu, Jiang; Zolg, Johannes W.  
PATENT ASSIGNEE(S): Millennium Pharmaceuticals, Inc., USA  
SOURCE: PCT Int. Appl., 182 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2005032328	A2	20050414	WO 2004-US15761	20040520
WO 2005032328	A3	20051215		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2005142569	A1	20050630	US 2004-849989	20040520
EP 1625235	A2	20060215	EP 2004-809388	20040520
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, PL, SK, HR				
PRIORITY APPLN. INFO.:			US 2003-472330P	P 20030521
			WO 2004-US15761	W 20040520

AB The authors disclose serum markers wherein changes in the levels of expression of one or more of the markers is correlated with RA.

L4 ANSWER 41 OF 53 HCPLUS COPYRIGHT 2008 ACS on STN  
ACCESSION NUMBER: 2005:29631 HCPLUS  
DOCUMENT NUMBER: 143:23559  
TITLE: Viable, apoptotic and necrotic monocytes expose phosphatidylserine: cooperative binding of the ligand Annexin V to dying but not viable cells and implications for PS-dependent clearance  
AUTHOR(S): Appelt, U.; Sheriff, A.; Gaipl, U. S.; Kalden, J. R.; Voll, R. E.; Herrmann, M.  
CORPORATE SOURCE: Institute for Clinical Immunology and Rheumatology, Department of Internal Medicine III, Friedrich-Alexander University of Erlangen-Nuernberg, Erlangen, 91054, Germany  
SOURCE: Cell Death and Differentiation (2005), 12(2), 194-196  
CODEN: CDDIEK; ISSN: 1350-9047  
PUBLISHER: Nature Publishing Group  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB The binding efficiencies of Annexin V (AxV) to phosphatidylserine on cells were investigated using vital and dying cells as PS bearer to simulate the in vivo situation. Anal. of whether AxV

shows pos. or neg. cooperativity for PS binding on membranes showed that AxV displays homotropic, pos. cooperativity upon binding to membranes, with high PS amts. as found in dying cells. This means that one AxV phys. interacts with another to change its conformation in such a way that it improves the binding abilities to PS. These data suggest a mechanism for phagocytes to differentiate dying and vital cells by means of PS clustering and/or lateral mobility due to membrane fluidity. This might explain why vital cells are not subject to phagocytosis.

REFERENCE COUNT: 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 42 OF 53 HCPLUS COPYRIGHT 2008 ACS on STN  
ACCESSION NUMBER: 2004:954318 HCPLUS  
DOCUMENT NUMBER: 142:22226  
TITLE: Inhibition of phosphatidylserine recognition heightens the immunogenicity of irradiated lymphoma cells in vivo  
AUTHOR(S): Bondanza, Attilio; Zimmermann, Valerie S.; Rovere-Querini, Patrizia; Turnay, Javier; Dumitriu, Ingrid E.; Stach, Christian M.; Voll, Reinhard E.; Gaipl, Udo S.; Bertling, Wolf; Poeschl, Ernst; Kalden, Joachim R.; Manfredi, Angelo A.; Herrmann, Martin  
CORPORATE SOURCE: Clinical Immunology Unit, Cancer Immunotherapy and Gene Therapy Program, H. San Raffaele Scientific, Institute and Vita-Salute University, Milan, 20132, Italy  
SOURCE: Journal of Experimental Medicine (2004), 200(9), 1157-1165  
CODEN: JEMEAV; ISSN: 0022-1007  
PUBLISHER: Rockefeller University Press  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Strategies to enhance the immunogenicity of tumors are urgently needed. Although vaccination with irradiated dying lymphoma cells recruits a tumor-specific immune response, its efficiency as immunogen is poor. Annexin V (AxV) binds with high affinity to phosphatidylserine on the surface of apoptotic and necrotic cells and thereby impairs their uptake by macrophages. Here, we report that AxV preferentially targets irradiated lymphoma cells to CD8+ dendritic cells for in vivo clearance, elicits the release of proinflammatory cytokines and dramatically enhances the protection elicited against the tumor. The response was endowed with both memory, because protected animals rejected living lymphoma cells after 72 d, and specificity, because vaccinated animals failed to reject unrelated neoplasms. Finally, AxV-coupled irradiated cells induced the regression of growing tumors. These data indicate that endogenous adjuvants that bind to dying tumor cells can be exploited to target tumors for immune rejection.

REFERENCE COUNT: 47 THERE ARE 47 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 43 OF 53 HCPLUS COPYRIGHT 2008 ACS on STN  
ACCESSION NUMBER: 2004:445662 HCPLUS  
DOCUMENT NUMBER: 141:49717  
TITLE: Assay of cell proliferation and apoptosis  
AUTHOR(S): Morii, Eiichi  
CORPORATE SOURCE: Grad. Sch. Med., Osaka Univ., Japan  
SOURCE: Byori to Rinsho (2004), 22(Rinjizokango, Byori Shindan ni okeru Bunshi Seibutsugaku), 364-370  
CODEN: BYRIEM; ISSN: 0287-3745  
PUBLISHER: Bunkodo  
DOCUMENT TYPE: Journal; General Review  
LANGUAGE: Japanese  
AB A review on methods for detection of cell proliferation by BrdU method, immunostaining by Ki-67 monoclonal antibodies, anti-topoisomerase

II $\alpha$  antibody, anti-PCNA antibody, etc., detection of apoptotic cells by TUNEL method, immunostaining of ss-DNA, activated caspase 3, 85-kDa fragment derived from PARP [poly(ADP-ribose) polymerase], etc., and phosphatidylserine-binding annexin V , and observation of DNA amount by flow cytometry.

L4 ANSWER 44 OF 53 HCPLUS COPYRIGHT 2008 ACS on STN  
ACCESSION NUMBER: 2004:182220 HCPLUS  
DOCUMENT NUMBER: 140:195470  
TITLE: Imaging cell death in vivo using non-radionuclide contrast agents  
INVENTOR(S): Montaldo, Michael; Johnson, Bruce; Amaratunga, Mohan  
PATENT ASSIGNEE(S): USA  
SOURCE: U.S. Pat. Appl. Publ., 9 pp.  
CODEN: USXXCO  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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US 2004042959	A1	20040304	US 2002-229689	20020828
PRIORITY APPLN. INFO.:			US 2002-229689	20020828

AB An annexin, annexin analog or phosphatidylserine binding compound (PSC) labeled with an MR, CT, or optical contrast agent aids in the detection of physiol. changes associated with tissue abnormalities such as cardiovascular disease, thrombosis and cancer. The conjugate is administered into a subject and specifically binds to the surface of apoptotic and necrotic cells. The subject is imaged using conventional MRI, CT and optical imaging techniques and dead and dying tissue is identified. The identification and development of analogs specific for phosphatidylserine for purposes of non-invasive imaging of dead or dying cells are described.

L4 ANSWER 45 OF 53 HCPLUS COPYRIGHT 2008 ACS on STN  
ACCESSION NUMBER: 2003:599641 HCPLUS  
DOCUMENT NUMBER: 140:141779  
TITLE: Optical imaging of apoptosis as a biomarker of tumor response to chemotherapy  
AUTHOR(S): Schellenberger, Eyk A.; Bogdanov, Alexei, Jr.; Petrovsky, Alexander; Ntziachristos, Vasilis; Weissleder, Ralph; Josephson, Lee  
CORPORATE SOURCE: Center for Molecular Imaging Research, Massachusetts General Hospital, Charlestown, MA, 02129, USA  
SOURCE: Neoplasia (Wilton, CT, United States) (2003), 5(3), 187-192  
CODEN: NEOPFL; ISSN: 1522-8002  
PUBLISHER: Neoplasia Press Inc.  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB A rapid and accurate assessment of the antitumor efficacy of new therapeutic drugs could speed up drug discovery and improve clin. decision making. Based on the hypothesis that most effective antitumor agents induce apoptosis, we developed a near-IR fluorescent (NIRF) annexin V to be used for optical sensing of tumor environments. To demonstrate probe specificity, we developed both an active (i.e., apoptosis-recognizing) and an inactive form of annexin V with very similar properties (to account for non-specific tumor accumulation), and tested the agents in nude mice each bearing a cyclophosphamide (CPA) chemosensitive (LLC) and a chemoresistant LLC (CR-LLC). After injection with active annexin V, the tumor-annexin V ratio (TAR; tumor NIRF/background NIRF) for untreated mice was 1.22 ± 0.34 for LLC and 1.43 ± 0.53 for

CR-LLC (n=4). The LLC of CPA-treated mice had significant elevations of TAR ( $2.56 \pm 0.29$ , P=.001, n=4), but only a moderate increase was obtained for the CR-LLC (TAR= $1.89 \pm 0.19$ , P=.183). The in vivo measurements correlated well with terminal deoxyribosyl transferase-mediated dUTP nick end labeling indexes. When inactive Cy-annexin V was used, with or without CPA treatment and in both LLC and CR-LLC tumors, tumor NIRF values ranged from 0.91 to 1.17 (i.e., tumor were equal to background). We conclude that active Cy-annexin V and surface reflectance fluorescence imaging provide a non-radioactive, semiquant. method of determining chemosensitivity in LLC xenografts. The method maybe used to image pharmacol. responses in other animal models and, potentially, may permit the clin. imaging of apoptosis with non-invasive or minimally invasive instrumentation.

REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 46 OF 53 HCPLUS COPYRIGHT 2008 ACS on STN  
 ACCESSION NUMBER: 2001:50517 HCPLUS  
 DOCUMENT NUMBER: 134:105841  
 TITLE: Cancer treatment using angiopoietins targeted to aminophospholipids  
 INVENTOR(S): Thorpe, Philip E.  
 PATENT ASSIGNEE(S): Maine Medical Center Research Institute, USA; Board of Regents, the University of Texas System  
 SOURCE: PCT Int. Appl., 248 pp.  
 CODEN: PIXXD2  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001003735	A1	20010118	WO 2000-US18779	20000711
W: AU, CA, JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				

PRIORITY APPLN. INFO.: US 1999-143762P P 19990712  
 AB Disclosed is the surprising discovery that aminophospholipids, such as phosphatidylserine and phosphatidylethanolamine, are specific, accessible and stable markers of the luminal surface of tumor blood vessels. The present invention particularly provides therapeutic constructs and conjugates that bind to aminophospholipids and contain angiopoietins, and various methods of specifically delivering angiopoietins to the stably-expressed aminophospholipids of tumor blood vessels, thereby exerting anti-tumor effects. The constructs can include binding ligands or antibodies and antibody fragments against the aminophospholipids. Pharmaceutical compns. and kits containing the targeting agent-angiopoietin constructs are also claimed; both the formulations and kit can also contain a second anticancer agent.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 47 OF 53 HCPLUS COPYRIGHT 2008 ACS on STN  
 ACCESSION NUMBER: 2000:321478 HCPLUS  
 DOCUMENT NUMBER: 132:331668  
 TITLE: Methods for the temporal analysis of programmed cell death in living cells using reagent having affinity for phosphatidylserine  
 INVENTOR(S): Maiese, Kenneth; Vincent, Andrea M.  
 PATENT ASSIGNEE(S): Wayne State University, USA  
 SOURCE: U.S., 16 pp., Cont.-in-part of U.S. Ser. No. 144,045.  
 CODEN: USXXAM  
 DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 3

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6063580	A	20000516	US 1999-275831	19990325
US 5939267	A	19990817	US 1998-144045	19980831
WO 2000013022	A1	20000309	WO 1999-US19767	19990827
W: CA, JP				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 1110087	A1	20010627	EP 1999-968262	19990827
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRIORITY APPLN. INFO.: US 1998-144045 A2 19980831  
US 1999-275831 A 19990325  
WO 1999-US19767 W 19990827

AB Methods for determining the induction and assessing the course of programmed cell death (PCD) over time in living cells are provided. The methods of the present invention comprise the steps of contacting viable cells with a detectable reagent having high affinity for phosphatidylserine, qual. and/or quant. detecting the cells that have reacted with the detectable reagent, removing the detectable reagent, recontacting the cells with the detectable reagent and qual. and/or quant. detecting cells that have reacted with the detectable reagent. The methods of the present invention are performed with cells maintained in a viable state, thereby allowing detection of the induction and assessment of the progression of PCD over time. Hippocampal neuronal cultures were treated with sodium nitroprusside, a NO generator, and stained for externalization of phosphatidylserine with annexin V conjugated to phycoerythrin. The annexin V was removed in Ca-free conditions. Neurons were examined by microscopy.

REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 48 OF 53 HCPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 2000:145996 HCPLUS

DOCUMENT NUMBER: 133:101543

TITLE: Detection of apoptosis in a heterogenous cell population using flow cytometry

AUTHOR(S): Sedlak, J.; Hunakova, L'; Duraj, J.; Sulikova, M.; Chovancova, J.; Novotny, L.; Chorvath, B.

CORPORATE SOURCE: Department of Molecular Immunology, Cancer Research Institute, Slovak Academy of Sciences, Bratislava, 833 91, Slovakia

SOURCE: General Physiology and Biophysics (1999), 18(Focus Issue), 147-154

CODEN: GPBIE2; ISSN: 0231-5882

PUBLISHER: Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Apoptosis induced in human leukemic cells (promyelocytic human leukemic cells HL-60, multidrug-resistant subline HL-60/VCR) and human ovarian carcinoma cells (A2780 and multidrug-resistant subline A2780/ADR) in vitro was detected by flow cytometric anal. or DNA electrophoresis. The cytofluorometric technique utilized, i.e. detection of phosphatidylserine exposed at the outer surface of the plasma membrane, identification of cells with "sub-G0" DNA content or increased light side scatter (cell internal structure) correlated with the electrophoretic determination of DNA fragmentation ("DNA ladder"). Detection of the 34 kDa mitochondrial protein recognized by the monoclonal antibody Apo2.7 yielded elevated percentages of apoptotic cells, suggesting that this technique detecting

both early and late apoptosis in digitonin-fixed cells might not be restricted to the specific detection of programmed cell death.

REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 49 OF 53 HCPLUS COPYRIGHT 2008 ACS on STN  
ACCESSION NUMBER: 1999:271483 HCPLUS  
DOCUMENT NUMBER: 130:293614  
TITLE: Green fluorescent protein-annexin fusion proteins with useful fluorescence and phospholipid binding properties  
INVENTOR(S): Ernst, Joel D.  
PATENT ASSIGNEE(S): The Regents of the University of California, USA  
SOURCE: PCT Int. Appl., 23 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9919470	A2	19990422	WO 1998-US21444	19981009
WO 9919470	A3	19990701		
W: AU, CA, JP				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 6511829	B1	20030128	US 1997-948276	19971009
AU 9897983	A	19990503	AU 1998-97983	19981009
EP 1021465	A2	20000726	EP 1998-952233	19981009
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2001520008	T	20011030	JP 2000-516023	19981009
PRIORITY APPLN. INFO.:			US 1997-948276	A 19971009
			WO 1998-US21444	W 19981009

AB Bifunctional green fluorescent protein (GFP)-annexin fusion proteins combine the inherent strong visible fluorescent properties of GFPs with the anionic phospholipid binding specificity of annexins. Recombinant host cells, especially bacteria, are used to efficiently express the fusion proteins in high yield and soluble form, suitable for rapid, one-step affinity purification. The endogenously fluorescent phosphatidylserine-binding proteins containing Aequorea victoria GFP fused to annexins offer highly sensitive detection of apoptotic cells by flow cytometry or fluorescent microscopy, and offer several advantages to chemical modified annexins. Uses include selective cellular and biochem. labeling, particularly anionic species, such as selectively labeling apoptotic cells.

L4 ANSWER 50 OF 53 HCPLUS COPYRIGHT 2008 ACS on STN  
ACCESSION NUMBER: 1999:5970 HCPLUS  
DOCUMENT NUMBER: 130:194787  
TITLE: Binding of annexin V to bilayers with various phospholipid compositions using glass beads in a flow cytometer  
AUTHOR(S): Stuart, M. C. A.; Reutelingsperger, C. P. M.; Frederik, P. M.  
CORPORATE SOURCE: EM Unit, Department of Pathology, University of Maastricht, Maastricht, 6200 MD, Neth.  
SOURCE: Cytometry (1998), 33(4), 414-419  
CODEN: CYTODQ; ISSN: 0196-4763  
PUBLISHER: Wiley-Liss, Inc.  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB Annexin V belongs to the family of calcium-dependent

phospholipid binding proteins and binds almost solely to phosphatidylserine (PS). When annexin V is used to detect loss of membrane asymmetry in cellular systems, the binding properties under physiol. conditions are of importance. Most biochem. studies use optimized binding conditions, conditions that are often far from physiol. For the interpretation of flow cytometric studies that use fluorescent annexin V to probe PS exposure, it is important to know what mixture of lipid species exposed in the outer leaflet of a membrane can evoke a pos. annexin V signal. The lipid species is important in this respect as well as the concentration that just

evokes a pos. signal (detection level). Furthermore, the influence of the composition of the lipid matrix (cholesterol content, other phospholipid species) was investigated, as well as the influence of the calcium concentration

on annexin V binding. In this study, we report on the binding of annexin V to phospholipid bilayers (adsorbed to glass beads) as measured by flow cytometry at physiol. conditions. Annexin V binding was found to increase rapidly, with increasing PS concns. up to a certain level (attained at 6 mol% PS). Further increase of the PS concentration resulted only in a slight increase of annexin V binding. Calcium concns. below 3 mM were found to reduce the sensitivity of the binding assay. Phosphatidylethanolamine incorporated in the phospholipid bilayer resulted in a lower threshold for the binding assay, whereas sphingomyelin had no influence on the binding of annexin V and cholesterol reduces binding of annexin V to lipid bilayers. These data may help in the interpretation of results obtained from binding of annexin V to cell membranes (e.g., involved in apoptosis).

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 51 OF 53 HCPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 1998:761669 HCPLUS

DOCUMENT NUMBER: 130:121137

TITLE: Phosphatidylserine exposure by apoptotic cells; a phylogenetically conserved mechanism

AUTHOR(S): Van Den Eijnde, Stefan M.; Boshart, Lenard; Baehrecke, Eric H.; Reutelingsperger, Chris P. M.; Vermeij-Keers, Christl

CORPORATE SOURCE: MGC Dep. Clin. Genetics, Erasmus Univ. Med. School, Rotterdam, 3000 DR, Neth.

SOURCE: Verhandelingen - Koninklijke Nederlandse Akademie van Wetenschappen, Afdeling Natuurkunde, Tweede Reeks (1998), 100(Pharmaceutical Intervention in Apoptotic Pathways), 63-73

CODEN: VNAWAG; ISSN: 0373-465X

PUBLISHER: North-Holland

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Tolerance of the existence of the individual cell in multicellular organisms is mediated by the distribution of the various phospholipid species across the bilayer of the plasma membrane. This concept arises from in vitro studies, which show that cell-surface exposed phosphatidylserine on aging erythrocytes and apoptotic leukocytes triggers elimination of these cells by phagocytosis. In contrast, blood cells are inert in this respect when this aminophospholipid is predominantly residing in the plasma membrane leaflet facing the cytoplasm. We have studied the in vivo distribution of cell surface-exposed phosphatidylserine by injecting biotinylated AnxV, a Ca<sup>2+</sup>-dependent phosphatidylserine binding protein, into viable mouse and chick embryos and Drosophila pupae. The apparent binding of Annexin V to cells that were present in regions of

developmental cell death and which exhibited the morphol. characteristic of apoptosis indicates that phosphatidylserine exposure by apoptotic cells is a phylogenetically conserved mechanism.

REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 52 OF 53 HCPLUS COPYRIGHT 2008 ACS on STN  
ACCESSION NUMBER: 1995:954152 HCPLUS  
DOCUMENT NUMBER: 124:5636  
TITLE: Binding and phagocytosis of apoptotic vascular smooth muscle cells is mediated in part by exposure of phosphatidylserine  
AUTHOR(S): Bennett, M. R.; Gibson, D. F.; Schwartz, S. M.; Tait, J. F.  
CORPORATE SOURCE: Dep. of Pathology, Univ. of Washington, Seattle, WA, USA  
SOURCE: Circulation Research (1995), 77(6), 1136-42  
CODEN: CIRUAL; ISSN: 0009-7330  
PUBLISHER: American Heart Association  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Apoptosis of vascular smooth muscle cells has recently been demonstrated to occur in vitro and in vivo. Uptake of apoptotic cells into adjacent normal cells appears to be rapid and specific. The authors have investigated binding and phagocytosis of apoptotic vascular smooth muscle cells by normal smooth muscle cell monolayers. Vascular smooth muscle cells were infected with the proto-oncogene c-myc or the adenovirus E1A gene, induced to undergo apoptosis in low-serum conditions, and then incubated with normal smooth muscle cells. Apoptosis was accompanied by a marked increase in exposure of phosphatidylserine in the outer surface of the cell, which was recognized by binding to annexin V. Liposomes containing phosphatidylserine but not phosphatidylinositol inhibited uptake of apoptotic cells in a dose-dependent manner to a maximum of 50% inhibition; annexin V also inhibited the uptake of apoptotic cells in a dose-dependent and calcium-dependent manner. Binding of apoptotic bodies did not appear to be mediated by endogenous annexin V, as evidenced by the inability of an antibody of annexin V to inhibit uptake. Smooth muscle cells were also able to recognize exposed phosphatidylserine on other cell types, as judged by their ability to bind erythrocytes having a high degree of exposed phosphatidylserine. The authors conclude that smooth muscle cells express phosphatidylserine during apoptosis, and this exposure partly mediates binding and phagocytosis of dead cells. This mechanism may be important in promoting rapid cell removal in the vessel wall.

L4 ANSWER 53 OF 53 HCPLUS COPYRIGHT 2008 ACS on STN  
ACCESSION NUMBER: 1994:502243 HCPLUS  
DOCUMENT NUMBER: 121:102243  
TITLE: Annexins possess functionally distinguishable Ca<sup>2+</sup> and phospholipid binding domains  
AUTHOR(S): Ernst, Joel D.; Mall, Alison; Chew, Gordon  
CORPORATE SOURCE: Dep. Med., Univ. California, San Francisco, CA, USA  
SOURCE: Biochemical and Biophysical Research Communications (1994), 200(2), 867-76  
CODEN: BBRCA9; ISSN: 0006-291X  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB All annexins bind Ca<sup>2+</sup> and phospholipids, although individual annexins differ markedly in their affinities for these ligands. Annexin I binds phosphatidylserine (PS) at lower [Ca+] than annexin V, while annexin V exhibits a higher affinity for PS than does annexin I. To identify the structural determinants of these properties, the authors characterized a

series of chimeric annexins. A chimera containing repeat 1 of annexin V fused to repeats 2, 3, and 4 of annexin I exhibited a Ca<sup>2+</sup> requirement for PS binding close to that of annexin, I, while chimeras containing repeat 1 of annexin I fused to repeats 2, 3, and 4 of annexin V required higher [Ca<sup>2+</sup>], similar to that of annexin V. In contrast, the overall affinity for APS vesicles was determined by the source of repeat 1. The chimera that contained repeat 1 of annexin V exhibited a high affinity for PS, while a chimera that contained repeat 1 of annexin I had a low affinity for PS similar to that of annexin I. The authors conclude that the [Ca<sup>2+</sup>] requirement for phospholipid binding and the overall phospholipid affinity of annexins are determined by distinct domains.

=> d his

(FILE 'HOME' ENTERED AT 12:11:46 ON 29 JAN 2008)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCPLUS, NTIS, LIFESCI' ENTERED AT 12:12:17 ON 29 JAN 2008

L1 628 S PHOSPHATIDYL SERINE(W) BIND?  
L2 162 S L1 AND ANNEXIN?  
L3 77 DUP REM L2 (85 DUPLICATES REMOVED)  
L4 53 S "ANNEXIN V" AND L3

=> s l1(w) protein?  
L5 131 L1(W) PROTEIN?

=> s kunitz  
L6 8823 KUNITZ

=> s 15 and 16  
L7 0 L5 AND L6

=> s l1 and coagulant?  
L8 3 L1 AND COAGULANT?

=> d 1-3 ibib ab

L8 ANSWER 1 OF 3 MEDLINE on STN  
ACCESSION NUMBER: 2004590568 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 15452129  
TITLE: Identification of the MMRN1 binding region within the C2 domain of human factor V.  
AUTHOR: Jeimy Samira B; Woram Rachael A; Fuller Nola; Quinn-Allen Mary Ann; Nicolaes Gerry A F; Dahlback Bjorn; Kane William H; Hayward Catherine P M  
CORPORATE SOURCE: Health Sciences Centre 2N31, Pathology and Molecular Medicine, McMaster University, 1200 Main Street West, Hamilton, Ontario L8N 3Z5, Canada.  
CONTRACT NUMBER: HL43106 (United States NHLBI)  
HL54939 (United States NHLBI)  
SOURCE: The Journal of biological chemistry, (2004 Dec 3) Vol. 279, No. 49, pp. 51466-71. Electronic Publication: 2004-09-27. Journal code: 2985121R. ISSN: 0021-9258.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
(RESEARCH SUPPORT, NON-U.S. GOV'T)  
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
OTHER SOURCE: REFSEQ-NP\_000121; REFSEQ-NP\_032002; REFSEQ-NP\_776304  
ENTRY MONTH: 200501

ENTRY DATE: Entered STN: 30 Nov 2004  
Last Updated on STN: 12 Jan 2005  
Entered Medline: 11 Jan 2005

AB In platelets, coagulation cofactor V is stored in complex with multimerin 1 in alpha-granules for activation-induced release during clot formation. The molecular nature of multimerin 1 factor V binding has not been determined, although multimerin 1 is known to interact with the factor V light chain. We investigated the region in factor V important for multimerin 1 binding using modified enzyme-linked immunoassays and recombinant factor V constructs. Factor V constructs lacking the C2 region or entire light chain had impaired and absent multimerin 1 binding, respectively, whereas the B domain deleted construct had modestly reduced binding. Analyses of point mutated constructs indicated that the multimerin 1 binding site in the C2 domain of factor V partially overlaps the phosphatidylserine binding site and that the factor V B domain enhances multimerin 1 binding. Multimerin 1 did not inhibit factor V phosphatidylserine binding, and it bound to phosphatidylserine independently of factor V. There was a reduction in factor V in complex with multimerin 1 after activation, and thrombin cleavage significantly reduced factor V binding to multimerin 1. In molar excess, multimerin 1 minimally reduced factor V procoagulant activity in prothrombinase assays and only if it was added before factor V activation. The dissociation of factor V-multimerin 1 complexes following factor V activation suggests a role for multimerin 1 in delivering and localizing factor V onto platelets prior to prothrombinase assembly.

L8 ANSWER 2 OF 3 HCAPLUS COPYRIGHT 2008 ACS on STN  
ACCESSION NUMBER: 2006:736541 HCAPLUS  
DOCUMENT NUMBER: 145:180946  
TITLE: Anti-phosphatidylserine immunoglobulin fusion products for use in targeting tumor neovascularization in cancer therapy  
INVENTOR(S): Thorpe, Philip E.; Luster, Troy A.; King, Steven W.  
PATENT ASSIGNEE(S): Board of Regents, The University of Texas System, USA; Peregrine Pharmaceuticals, Inc.  
SOURCE: PCT Int. Appl., 361 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2006079120	A2	20060727	WO 2006-US2964	20060124
WO 2006079120	A3	20070719		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AP, EA, EP, OA				
AU 2006206187	A1	20060727	AU 2006-206187	20060124
CA 2591914	A1	20060727	CA 2006-2591914	20060124
US 2006228299	A1	20061012	US 2006-339392	20060124
EP 1853631	A2	20071114	EP 2006-719706	20060124
R: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LI, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, AL,				

BA, HR, MK, YU  
 IN 2007DN05739 A 20070817 IN 2007-DN5739 20070724  
 PRIORITY APPLN. INFO.: US 2005-646333P P 20050124  
 WO 2006-US2964 W 20060124

**AB** Fusion proteins of phosphatidylserine-binding proteins that have surprising combinations of properties, and a range of diagnostic and therapeutic uses are described. The new constructs effectively bind phosphatidylserine targets in disease and enhance their destruction, and can also specifically deliver attached imaging or therapeutic agents to the disease site. Also disclosed are methods of using the new construct compns., therapeutic conjugates and combinations thereof in tumor vasculature targeting, cancer diagnosis and treatment, and for treating viral infections and other diseases. External phosphatidylserine is shown to be marker of blood vessels in tumors, and absent from normal tissues, and so can be used as a marker for diagnosis and in the targeting of tumor neovascularization in cancer therapy. Characterization of anti-phosphatidylserine antibodies and their use in targeting tumor neovascularization is reported. The antibodies are also useful in the treatment of enveloped viruses that present phosphatidylserine.

L8 ANSWER 3 OF 3 HCPLUS COPYRIGHT 2008 ACS on STN  
 ACCESSION NUMBER: 2000:53434 HCPLUS  
 DOCUMENT NUMBER: 132:106961  
 TITLE: Cancer treatment methods using therapeutic conjugates that bind to aminophospholipids  
 INVENTOR(S): Thorpe, Philip E.; Ran, Sophia  
 PATENT ASSIGNEE(S): Board of Regents, the University of Texas System, USA  
 SOURCE: PCT Int. Appl., 266 pp.  
 CODEN: PIXXD2  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000002587	A1	20000120	WO 1999-US15668	19990712
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2331789	A1	20000120	CA 1999-2331789	19990712
AU 9950958	A	20000201	AU 1999-50958	19990712
AU 750414	B2	20020718		
BR 9912053	A	20010403	BR 1999-12053	19990712
EP 1098665	A1	20010516	EP 1999-935491	19990712
EP 1098665	B1	20030108		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
US 6312694	B1	20011106	US 1999-351457	19990712
JP 2002520297	T	20020709	JP 2000-55846	19990712
AT 230614	T	20030115	AT 1999-935491	19990712
ES 2188202	T3	20030616	ES 1999-935491	19990712
NZ 508873	A	20031031	NZ 1999-508873	19990712
US 6818213	B1	20041116	US 1999-351598	19990712
US 7067109	B1	20060627	US 1999-351149	19990712
MX 2001PA00455	A	20010521	MX 2001-PA455	20010112
US 6783760	B1	20040831	US 2001-819386	20010328
HK 1038498	A1	20040116	HK 2001-108089	20011116
US 2005089523	A1	20050428	US 2004-988245	20041112

US 2006083745	A1	20060420	US 2005-254137	20051019
PRIORITY APPLN. INFO.:			US 1998-92589P	P 19980713
			US 1998-110600P	P 19981202
			US 1999-351149	A1 19990712
			US 1999-351457	A3 19990712
			US 1999-351598	A1 19990712
			WO 1999-US15668	W 19990712

AB Disclosed is the surprising discovery that aminophospholipids, such as phosphatidylserine and phosphatidylethanolamine, are specific, accessible and stable markers of the luminal surface of tumor blood vessels. The present invention thus provides aminophospholipid-targeted diagnostic and therapeutic constructs for use in tumor intervention. Antibody-therapeutic agent conjugates and constructs that bind to aminophospholipids are particularly provided, as are methods of specifically delivering therapeutic agents, including toxins and coagulants, to the stably-expressed aminophospholipids of tumor blood vessels, thereby inducing thrombosis, necrosis and tumor regression.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d his

(FILE 'HOME' ENTERED AT 12:11:46 ON 29 JAN 2008)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 12:12:17 ON 29 JAN 2008

L1	628 S PHOSPHATIDYLSERINE(W) BIND?
L2	162 S L1 AND ANNEXIN?
L3	77 DUP REM L2 (85 DUPLICATES REMOVED)
L4	53 S "ANNEXIN V" AND L3
L5	131 S L1(W) PROTEIN?
L6	8823 S KUNITZ
L7	0 S L5 AND L6
L8	3 S L1 AND COAGULANT?

=> s antocoagulant and l1  
L9 0 ANTICOAGULANT AND L1

=> s anticoagulant and l1  
L10 21 ANTICOAGULANT AND L1

=> dup rem l10  
PROCESSING COMPLETED FOR L10  
L11 16 DUP REM L10 (5 DUPLICATES REMOVED)

=> d 1-16 ibib ab

L11	ANSWER 1 OF 16 HCAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER:	2007:63346 HCAPLUS
DOCUMENT NUMBER:	146:159824
TITLE:	Modified annexin proteins and methods for their use in platelet storage and transfusion
INVENTOR(S):	Allison, Anthony Clifford
PATENT ASSIGNEE(S):	USA
SOURCE:	U.S. Pat. Appl. Publ., 78pp., Cont.-in-part of U.S. Ser. No. 267,837.
DOCUMENT TYPE:	Patent
LANGUAGE:	English
FAMILY ACC. NUM. COUNT:	7
PATENT INFORMATION:	

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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US 2007015705	A1	20070118	US 2006-486667	20060714
US 2003166532	A1	20030904	US 2002-80370	20020221
US 6962903	B2	20051108		
EP 1839670	A2	20071003	EP 2007-106226	20020221
R: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE, TR				
US 2005222030	A1	20051006	US 2005-78231	20050310
US 2005197295	A1	20050908	US 2005-110306	20050420
US 2006105952	A1	20060518	US 2005-267837	20051103
WO 2008008561	A2	20080117	WO 2007-US66561	20070412
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW				
RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
WO 2008008982	A2	20080117	WO 2007-US73507	20070713
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW				
RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				

PRIORITY APPLN. INFO.:

US 2001-270402P	P 20010221
US 2001-332582P	P 20011121
US 2002-80370	A2 20020221
US 2004-552428P	P 20040311
US 2004-579589P	P 20040614
US 2005-78231	A2 20050310
US 2005-267837	A2 20051103
EP 2002-721083	A3 20020221
US 2006-486667	A 20060714

AB Modified annexin proteins, including homodimers of human annexin V, IV, or VIII are provided. Methods for their use are also provided, such as to prevent thrombosis without increasing hemorrhage, enhancing the survivability of platelets during storage or transfusion, and to attenuate ischemia-reperfusion injury (IPI). The modified annexins bind phosphatidylserine (PS) on cell surfaces, thereby preventing the assembly of the prothrombinase complex. The modified annexin decreases the binding of leukocytes and platelets during post-ischemic reperfusion, thereby restoring microvascular blood flow and decreasing organ damage. In addition, the modified annexin prevents lipid loss from platelets during storage.

L11 ANSWER 2 OF 16 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 2006-23099 BIOTECHDS

TITLE: Inducing hemostasis or thrombosis in an animal comprises administering a chimeric protein comprising a phosphatidylserine binding domain and a soluble tissue factor domain having human Tissue Factor activity to the animal; involving vector-mediated gene transfer and expression in

host cell fpr use in telangiectasia, arterial malformation, venous malformation, capillary malformation, lymphatic malformation, arterio-venous malformation, hemangioma and aneurysm therapy

AUTHOR: LIND S E; DING W; HARRISON R G  
PATENT ASSIGNEE: LIND S E; DING W; HARRISON R G  
PATENT INFO: WO 2006096828 14 Sep 2006  
APPLICATION INFO: WO 2006-US8541 9 Mar 2006  
PRIORITY INFO: US 2005-659938 9 Mar 2005; US 2005-659938 9 Mar 2005  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 2006-635900 [66]

AB DERWENT ABSTRACT:

NOVELTY - Inducing hemostasis or thrombosis in an animal comprises providing a chimeric protein comprising a phosphatidylserine binding domain and a soluble tissue factor domain comprising fully defined 217 amino acids (SEQ ID NO. 2) or its mutant or portion having human Tissue Factor activity; and administering the chimeric protein to the animal in an amount to promote coagulation in a vascularized area of the animal.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a medicine or composition for inducing hemostasis or thrombosis in an animal comprising a chimeric protein comprising a soluble tissue factor domain comprising SEQ ID NO. 2, or its mutant or portion having human tissue factor activity; and a phosphatidyl serine binding domain, and where the chimeric protein is disposed in a carrier.

BIOTECHNOLOGY - Preferred Method: In inducing hemostasis or thrombosis in an animal, the vascularized area of the animal is non-cancerous. The phosphatidylserine-binding domain comprises an Annexin, preferably human Annexin V. The chimeric protein mutant of SEQ ID NO. 2 comprises one substitution selected from Ala at position 13, 131, 163, 164, or 183; Asn at position 42 or 138; Trp at position 48; Ser at position 52; Asp at position 128; Gln at position 129, 163, or 164; and Glu at position 163 or 164. The chimeric protein further comprises Ser-Gly or Gly at the amino-terminus of SEQ ID NO. 2. It further comprises a linker connecting the soluble tissue factor domain and the phosphatidylserine-binding domain. The vascularized area of the animal is bleeding due to presence of an anticoagulant drug; a lack of a coagulation factor due to trauma, transfusion, antibodies, or congenital conditions; liver disease; vascular or head injury; gastrointestinal conditions including gastritis, ulcer, and esophageal varices; cystitis, endometritis; or bleeding due to insufficient platelets or improperly functioning platelets. The method further comprises administering the chimeric protein with recombinant Factor VIIa. The vascularized area comprises a telangiectasia, arterial malformation, venous malformation, capillary malformation, lymphatic malformation, arterio-venous malformation, hemangioma, or aneurysm. The method further comprises administering a drug, chemical, electrical stimulus or radiation in adjunct to the step of administering the chimeric protein. The chimeric protein further comprises a therapeutic compound or material where the chimeric protein serves to deliver the therapeutic compound or material to a site in need of treatment. The vascularized area to which the therapeutic compound or material is delivered is a tumor or abnormal vascular bed. Preferably, the therapeutic material or compound is chemotherapeutic or radioactive. Preferred Composition: The medicine or composition is directed to treating a vascularized area in the animal, where the vascularized area of the animal is bleeding due to presence of an anticoagulant drug; a lack of a coagulation factor due to trauma, transfusion, antibodies, or congenital conditions; liver disease; vascular or head injury; gastrointestinal conditions including gastritis, ulcer, and esophageal varices; cystitis, endometritis; or bleeding due to insufficient platelets or improperly functioning platelets or comprises a telangiectasia, arterial malformation, venous malformation, capillary

malformation, lymphatic malformation, arterio-venous malformation, hemangioma, or aneurysm.

ACTIVITY - Hemostatic; Thrombolytic; Vasotropic; Cytostatic. No biological data given.

MECHANISM OF ACTION - None given.

USE - The method is useful for inducing hemostasis or thrombosis in an animal. The medicine or composition is useful for treating a vascularized area in the animal, where the vascularized area comprises a telangiectasia, arterial malformation, venous malformation, capillary malformation, lymphatic malformation, arterio-venous malformation, hemangioma, or aneurysm.

ADMINISTRATION - The chimeric protein is administered locally in order to induce a local thrombosis (claimed). Dosage is 0.1 ng/kg -100 mg/kg, preferably 100 ng/kg - 10 mg/kg. Administration can be through oral, parenteral (e.g. subcutaneous, intravenous, intramuscular, intraperitoneal, or intratracheal), intraocular, or intracranial route.

EXAMPLE - No suitable example given.(60 pages)

L11 ANSWER 3 OF 16 HCAPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 2005:1026904 HCAPLUS  
DOCUMENT NUMBER: 143:299114  
TITLE: Modified annexin proteins and methods for preventing thrombosis  
INVENTOR(S): Allison, Anthony  
PATENT ASSIGNEE(S): Alavita, Inc., USA  
SOURCE: PCT Int. Appl., 95 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 7  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2005086955	A2	20050922	WO 2005-US8193	20050310
WO 2005086955	A3	20060309		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2005221195	A1	20050922	AU 2005-221195	20050310
CA 2559167	A1	20050922	CA 2005-2559167	20050310
EP 1734983	A2	20061227	EP 2005-725390	20050310
R: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LI, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR				
PRIORITY APPLN. INFO.:				
		US 2004-552428P	P	20040311
		US 2004-579589P	P	20040614
		WO 2005-US8193	W	20050310

AB A modified annexin protein, preferably annexin V, is used to prevent thrombosis without increasing hemorrhage. Annexin binds to phosphatidylserine on the outer surface of cell membranes, thereby preventing binding of the prothrombinase complex necessary for thrombus formation. It does not, however, affect platelet aggregation necessary for hemostasis. The modified annexin mol. can be a homodimer of annexin of an annexin mol. coupled to one or more polyethylene glycol chains. By increasing the mol. weight of annexin, the modified annexin is made to remain in circulation for sufficient time to provide a sustained therapeutic

effect.

L11 ANSWER 4 OF 16 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on  
STN

ACCESSION NUMBER: 2005:493160 SCISEARCH  
THE GENUINE ARTICLE: 921WW

TITLE: MBP-annexin V radiolabeled directly with iodine-124 can be used to image apoptosis in vivo using PET

AUTHOR: Dekker B; Keen H; Lyons S; Disley L; Hastings D; Reader A; Ottewell P; Watson A; Zweit J (Reprint)

CORPORATE SOURCE: UMIST, Canc Res UK, Dept Radiochem Targeting & Imaging, Paterson Inst Canc Res, Manchester M20 4BX, Lancs, England (Reprint); Univ Manchester, Inst Sci & Technol, Dept Instrumentat & Analyt Sci, Manchester M60 1QD, Lancs, England; Christie Hosp NHS Trust, Manchester M20 4BX, Lancs, England; Univ Liverpool, Dept Med, Liverpool L69 3GA, Merseyside, England  
jzweit@picr.man.ac.uk

COUNTRY OF AUTHOR: England

SOURCE: NUCLEAR MEDICINE AND BIOLOGY, (APR 2005) Vol. 32, No. 3, pp. 241-252.

ISSN: 0969-8051.

PUBLISHER: ELSEVIER SCIENCE INC, 360 PARK AVE SOUTH, NEW YORK, NY 10010-1710 USA.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 57

ENTRY DATE: Entered STN: 22 May 2005  
Last Updated on STN: 22 May 2005

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB A noninvasive method of measuring programmed cell death in the tumors of cancer patients using positron-emission tomography (PET) would provide valuable information regarding their response to therapeutic intervention. Our strategy is to radiolabel annexin V, a protein that binds to phosphatidylserine moieties that are translocated to the external leaflet of plasma membranes during apoptosis. We developed a phosphatidylserine-ELISA capable of distinguishing wild type from point mutant annexin V that is known to have a lower phosphatidylserine binding affinity. A maltose-binding protein/annexin V chimera was synthesized and purified with high yield using amylose resin.

We showed that it bound to phosphatidylserine in the ELISA as well as to that exposed on apoptotic Jurkat cells; therefore, it was used in the development of a method for radiolabeling annexin V using iodine radionuclides. MBP-annexin V retained its phosphatidylserine binding properties on direct iodination, but at high levels of oxidizing agents (iodogen and chloramine T), its specificity for phosphatidylserine was compromised. I-124-MBP-annexin V was successfully used to image Fas-mediated hepatic cell death in BDF-1 mice using PET.

In conclusion, we have shown that MBP-annexin V and the phosphatidylserine ELISA are useful tools for the development of methods for radiolabeling annexin V for PET imaging. (c) 2005 Elsevier Inc. All rights reserved.

L11 ANSWER 5 OF 16 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on  
STN

ACCESSION NUMBER: 2004:1077420 SCISEARCH  
THE GENUINE ARTICLE: 876JT

TITLE: Cell surface-expressed phosphatidylserine and annexin A5 open a novel portal of cell entry

AUTHOR: Kenis H; van Genderen H; Bennaghmouch A; Rinia H A; Frederik P; Narula J; Hofstra L; Reutelingsperger C P M (Reprint)

CORPORATE SOURCE: Univ Limburg, Cardiovasc Res Inst Maastricht, Dept Biochem, POB 616, NL-6200 MD Maastricht, Netherlands

(Reprint); Univ Limburg, Cardiovasc Res Inst Maastricht, Dept Biochem, NL-6200 MD Maastricht, Netherlands; Univ Limburg, Cardiovasc Res Inst Maastricht, Dept Cardiol, NL-6200 MD Maastricht, Netherlands; Univ Limburg, Cardiovasc Res Inst Maastricht, Dept Electron Microscopy, NL-6200 MD Maastricht, Netherlands; Leiden Inst Phys, NL-2333 CA Leiden, Netherlands; Univ Calif Irvine, Dept Med Cardiol, Irvine, CA 92612 USA  
c.reutelingsperger@bioch.unimaas.nl

COUNTRY OF AUTHOR: Netherlands; USA  
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (10 DEC 2004) Vol. 279, No. 50, pp. 52623-52629.

PUBLISHER: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3996 USA.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 36

ENTRY DATE: Entered STN: 6 Jan 2005

Last Updated on STN: 6 Jan 2005

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Expression of phosphatidylserine (PtdSer) at the cell surface is part of the membrane dynamics of apoptosis. Expressed phosphatidylserine functions as an "eat me" flag toward phagocytes. Here, we report that the expressed phosphatidylserine forms part of a hitherto undescribed pinocytic pathway. Annexin A5, a phosphatidylserine-binding protein, binds to and polymerizes through protein-protein interactions on membrane patches expressing phosphatidylserine. The two-dimensional protein network of annexin A5 at the surface prevents apoptotic body formation without interfering with the progression of apoptosis as demonstrated by activation of caspase-3, PtdSer exposure, and DNA fragmentation. The annexin A5 protein network bends the membrane patch nanomechanically into the cell and elicits budding, endocytic vesicle formation, and cytoskeleton-dependent trafficking of the endocytic vesicle. Annexin A1, which binds to PtdSer without forming a two-dimensional protein network, does not induce the formation of endocytic vesicles. This novel pinocytic pathway differs from macropinocytosis, which is preceded by membrane ruffling and actin polymerization. We clearly showed that actin polymerization is not involved in budding and endocytic vesicle formation but is required for intracellular trafficking. The phosphatidylserine-annexin A5-mediated pinocytic pathway is not restricted to cells in apoptosis. We demonstrated that living tumor cells can take up substances through this novel portal of cell entry. This opens new avenues for targeted drug delivery and cell entry.

L11 ANSWER 6 OF 16 MEDLINE on STN DUPLICATE 1  
ACCESSION NUMBER: 2004561292 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 15533308  
TITLE: Lactadherin binds selectively to membranes containing phosphatidyl-L-serine and increased curvature.  
AUTHOR: Shi Jialan; Heegaard Christian W; Rasmussen Jan T; Gilbert Gary E  
CORPORATE SOURCE: Department of Medicine, VA Boston Healthcare System, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02132, USA.  
CONTRACT NUMBER: R01 HL57867 (United States NHLBI)  
SOURCE: Biochimica et biophysica acta, (2004 Nov 17) Vol. 1667, No. 1, pp. 82-90.  
Journal code: 0217513. ISSN: 0006-3002.  
PUB. COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
(RESEARCH SUPPORT, NON-U.S. GOV'T)  
(RESEARCH SUPPORT, U.S. GOV'T, NON-P.H.S.)

(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)

LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200501  
ENTRY DATE: Entered STN: 10 Nov 2004  
Last Updated on STN: 22 Jan 2005  
Entered Medline: 21 Jan 2005

AB Lactadherin, a milk protein, contains discoidin-type lectin domains with homology to the phosphatidylserine-binding domains of blood coagulation factor VIII and factor V. We have found that lactadherin functions, *in vitro*, as a potent anticoagulant by competing with blood coagulation proteins for phospholipid binding sites [J. Shi and G.E. Gilbert, Lactadherin inhibits enzyme complexes of blood coagulation by competing for phospholipid binding sites, *Blood* 101 (2003) 2628-2636]. We wished to characterize the membrane-binding properties that correlate to the anticoagulant capacity. We labeled bovine lactadherin with fluorescein and evaluated binding to membranes of composition phosphatidylserine/phosphatidylethanolamine/phosphatidylcholine, 4:20:76 supported by 2 μm diameter glass microspheres. Lactadherin bound saturably with an apparent KD of 3.3+/-0.4 nM in a Ca<sup>++</sup> -independent manner. The number of lactadherin binding sites increased proportionally to the phosphatidylserine content over a range 0-2% and less rapidly for higher phosphatidylserine content. Inclusion of phosphatidylethanolamine in phospholipid vesicles did not enhance the apparent affinity or number of lactadherin binding sites. The number of sites was at least 4-fold higher on small unilamellar vesicles than on large unilamellar vesicles, indicating that lactadherin binding is enhanced by membrane curvature. Lactadherin bound to membranes with synthetic dioleoyl phosphatidyl-L-serine but not dioleoyl phosphatidyl-D-serine indicating stereoselective recognition of phosphatidyl-L-serine. We conclude that lactadherin resembles factor VIII and V with stereoselective preference for phosphatidyl-L-serine and preference for highly curved membranes.

L11 ANSWER 7 OF 16 HCPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 2005:1059796 HCPLUS  
DOCUMENT NUMBER: 143:419418  
TITLE: Binding activity of annexin B1 to externalized phosphatidylserine  
AUTHOR(S): Wang, Fang; Zhang, Yi; Yan, Hongli; Gao, Yuanjian;  
Liu, Fan; He, Yan; Sun, Shuhan  
CORPORATE SOURCE: Department of Medical Genetics, College of Basic  
Medical Sciences, Second Military Medical University,  
Shanghai, 200433, Peop. Rep. China  
SOURCE: Dier Junyi Daxue Xuebao (2004), 25(1), 44-46  
CODEN: DJXUE5; ISSN: 0258-879X  
PUBLISHER: Dier Junyi Daxue Xuebao Bianjibu  
DOCUMENT TYPE: Journal  
LANGUAGE: Chinese

AB The binding activity of annexin B1 to externalized phosphatidylserine was studied. Annexin B1 was expressed in *E. coli* and purified with ion-exchange chromatog., then annexin B1 labeled with FITC was used to detect the apoptosis of U937 human leukemic cells and the activation of platelets, which were analyzed by Becton Dickinson FACS Calibur. Labeled annexin B1 not only bound to apoptotic cells but also specifically activated platelets. Considering the affinity, annexin B1 was similar to annexin V. Annexin B1 interacts strongly and specifically with phosphatidylserine (PS) on the outer plasma membrane leaflet of apoptotic cells and activates platelets. Addnl., annexin B1 acts as a potent anticoagulant by binding to platelet membranous phospholipids with higher affinity.

L11 ANSWER 8 OF 16 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2003-19583 BIOTECHDS  
TITLE: New expression vector for producing annexin V, useful as

anticoagulant, includes kanamycin resistance gene,  
also Escherichia coli containing the vector;  
vector-mediated gene transfer and expression in  
Escherichia coli for recombinant protein production

AUTHOR: GREIN S; REISER C  
PATENT ASSIGNEE: NOVEMBER GES MOLEKULARE MED AG  
PATENT INFO: WO 2003052094 26 Jun 2003  
APPLICATION INFO: WO 2002-EP14347 16 Dec 2002  
PRIORITY INFO: DE 2001-1062434 18 Dec 2001; DE 2001-1062434 18 Dec 2001  
DOCUMENT TYPE: Patent  
LANGUAGE: German  
OTHER SOURCE: WPI: 2003-505487 [47]

AB DERWENT ABSTRACT:

NOVELTY - Expression vector (A) for production of annexin V (I), is new.  
DETAILED DESCRIPTION - Expression vector (A) comprising: (a) at least the components of pBR322 or pKK223-3 that are required for replication; (b) an expression region comprising, in sequence in the reading direction, a promoter (P1), a repressor-specific binding site (X), a prokaryotic ribosome-binding site (RBS), cloning site for the gene for annexin V (I), and at least one transcriptional terminator; and (c) at least one kanamycin resistance gene controlled by promoter P2. An INDEPENDENT CLAIM is also included for Escherichia coli cells that contain (A).

BIOTECHNOLOGY - Preferred Materials: The gene for (I) is from chicken and codons are optimized for expression in E. coli. The 3'-end of the gene has at least 1, preferably 2, additional stop codons, in reading frame. P1 is the tac promoter; the repressor is the lac repressor and the transcriptional terminator is rrnB T1 or T2. The components of (a) lack antibiotic resistance genes. The kanamycin resistance gene is from vector pACYC177 or the E. coli transposon Tn903; P2 is pK from vector pACYC177, and is inserted into EcoRI-StyI sites in pBR322 or EcoRI sites in pKK223-3. Most preferably the gene/P2 component comprises nucleotides 1816-2771 of pACYC177. The expression region is cloned into BamI-EcoRI sites in pBR322 and, without the annexin V gene, is a 181 base pair sequence (S1), given in the specification. (A) has no cleavage sites, outside the cloning site, for class II restriction endonucleases that include ATG in their recognition site, specifically NdeI and NcoI. Particularly (A), without the annexin V gene has a 3071 base pair sequence, and with this gene inserted has either a 4044 base pair sequence (S3), from pBR322, or a 5873 base pair sequence (S4), from pKK223-3, all given in the specification. Preparation: The vectors are produced by essentially conventional assembly of components. Preferred Cells: The E. coli host cells are of strain BL21 or a lacIq strain, particularly JM105.

ACTIVITY - Anticoagulant. No biological data is given.

MECHANISM OF ACTION - (I) binds to phosphatidylserine.

USE - (I) is used to inhibit coagulation of blood.

ADVANTAGE - The vector produces annexin V that is free of tetracycline or penicillin (which may cause anaphylactic shock) and contains only a non-toxic amount of kanamycin.

EXAMPLE - A fragment (nucleotides 1816-2771), containing the kanamycin resistance gene and attached pK promoter, was amplified from pACYC177 and cloned into pBR322, cut with EcoRI and StyI. The product was subjected to targeted mutation to eliminate a NdeI site, forming plasmid pBRK. Separately, a synthetic expression region was assembled by amplifying cDNA for annexin V (from chicken liver) then digestion with NdeI and BamHI and the product cloned into the corresponding enzyme sites in pBRK, between the Shine-Dalgarno site and the transcription terminator, to form an expression vector. (33 pages)

L11 ANSWER 9 OF 16 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN  
ACCESSION NUMBER: 2003:335602 BIOSIS  
DOCUMENT NUMBER: PREV200300335602  
TITLE: Lactadherin Inhibits Enzyme Complexes of Blood Coagulation

AUTHOR(S): by Competing for Phospholipid Binding Sites.  
Shi, Jialan [Reprint Author]; Gilbert, Gary E. [Reprint Author]

CORPORATE SOURCE: Medicine, VA Hospital, Brigham and Women's Hospital,  
Harvard Medical School, Boston, MA, USA

SOURCE: Blood, (November 16 2002) Vol. 100, No. 11, pp. Abstract  
No. 1002. print.

Meeting Info.: 44th Annual Meeting of the American Society  
of Hematology. Philadelphia, PA, USA. December 06-10, 2002.  
American Society of Hematology.

CODEN: BLOOAW. ISSN: 0006-4971.

DOCUMENT TYPE: Conference; (Meeting)  
Conference; (Meeting Poster)  
Conference; Abstract; (Meeting Abstract)

LANGUAGE: English

ENTRY DATE: Entered STN: 23 Jul 2003  
Last Updated on STN: 22 Aug 2003

AB Lactadherin, a glycoprotein of the milk fat globule membrane, contains tandem domains with homology to discoidin-type lectins and to membrane-binding "C" domains of blood-clotting factors V and VIII. We asked whether the structural homology confers the capacity to compete for the membrane-binding sites of factor VIII and factor V and to function as an anticoagulant. Therefore, we evaluated the capacity of lactadherin to compete with factors V and VIII for membrane binding and function. Bovine lactadherin, from which milk lipids had been quantitatively removed, stained as a doublet of glycosylation isoforms approx. Mr 47,000/50,000 with no other bands visible by SDS-PAGE with silver staining. Lactadherin competed efficiently with 4 nM fluorescein-labeled factor VIII for binding sites on phosphatidylserine-containing phospholipid bilayers (phosphatidylserine:phosphatidylethanolamine:phosphatidylcholine 4:20:76) supported by glass microspheres. Half-maximal competition occurred at 1.5 nM lactadherin with >95% competition at 16 nM. Lactadherin competed similarly with 4 nM fluorescein-labeled factor V with 50% competition at 3 nM lactadherin. Lactadherin inhibited the factor Xase complex (factor IXa - 0.1 nM, factor VIIIa - 1 nM, factor X - 100 nM) on sonicated vesicles (1 μM phospholipid) of the same composition with 50% inhibition at 6 nM lactadherin and 99% inhibition at 32 nM. In contrast, annexin V competed for fewer than 25% of the binding sites for factor VIII or factor V at concentrations up to 32 nM. Experiments with membranes of sonicated vs. extruded vesicles, with varying phosphatidylserine content and curvature, indicated that lactadherin was an efficient inhibitor on all membrane tested, causing >95% inhibition of both the factor Xase and prothrombinase complexes, while annexin V was only an effective inhibitor (>80%) on membranes containing high PS content and minimal curvature. Lactadherin also inhibited the factor VIIa-tissue factor complex at 2-4 fold higher concentrations than the factor Xase and prothrombinase complexes and competed with fluorescein-labeled factor IXa for phospholipid binding sites. To determine whether the phospholipid-blocking function is relevant in the complex milieu of whole blood, lactadherin was evaluated in whole blood with clotting initiated by 50 pM relipidated tissue factor. Lactadherin, 60 nM, prolonged the clotting time 150% vs. 20% for 60 nM annexin V. These results indicate that lactadherin is able to compete for phospholipid binding sites of factor VIII and factor V, as well as vitamin K-dependent proteins and can function as a potent phospholipid-blocking anticoagulant.

L11 ANSWER 10 OF 16 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on  
STN

ACCESSION NUMBER: 1997:907582 SCISEARCH

THE GENUINE ARTICLE: YK151

TITLE: In situ detection of apoptosis during embryogenesis with Annexin V: From whole mount to ultrastructure

AUTHOR: vandenEijnde S M (Reprint); Luijsterburg A J M; Boshart L;

DeZeeuw C I; vanDierendonck J H; Reutelingsperger C P M;  
VermeijKeers C  
CORPORATE SOURCE: ERASMUS UNIV ROTTERDAM, SCH MED, MGC, DEPT CLIN GENET, POB 1738, NL-3000 DR ROTTERDAM, NETHERLANDS (Reprint); ERASMUS UNIV ROTTERDAM, SCH MED, DEPT ANAT, ROTTERDAM, NETHERLANDS; ERASMUS UNIV ROTTERDAM, SCH MED, DEPT PLAST & RECONSTRUCT SURG, ROTTERDAM, NETHERLANDS; LEIDEN UNIV, MED CTR, DEPT SURG, LEIDEN, NETHERLANDS; UNIV MAASTRICHT, CARDIOVASC RES INST, DEPT BIOCHEM, MAASTRICHT, NETHERLANDS  
COUNTRY OF AUTHOR: NETHERLANDS  
SOURCE: CYTOMETRY, (1 DEC 1997) Vol. 29, No. 4, pp. 313-320.  
ISSN: 0196-4763.  
PUBLISHER: WILEY-LISS, DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English  
REFERENCE COUNT: 51  
ENTRY DATE: Entered STN: 1997  
Last Updated on STN: 1997

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Apoptosis is of paramount importance during embryonic development. This insight stems from early studies which correlated cell death to normal developmental processes and now has been confirmed by linking aberrant cell death patterns to aberrant development. Linking apoptosis to the phenotype of a developing organism requires spatial information on the localization of the dying cells, making *in situ* detection essential. This prerequisite limits the tools available for such studies (1) to vital dyes, which can be detected at the whole mount level only; (2) to detection based upon apoptotic morphology by routine light microscopy and electron microscopy; and (3) to staining for apoptosis associated DNA fragmentation via, e.g., the TUNEL procedure, which marks cells in a relative late phase of apoptosis. New apoptosis markers need to be specific and should preferably detect cells early during this process. In the present study we show that the recently discovered *in vitro* marker of apoptosis, Annexin V meets these requirements for *in vivo* detection. Through intracardiac injections of biotin labeled Annexin V, a Ca<sup>2+</sup> dependent phosphatidylserine binding protein, we were able to visualize apoptotic cells derived from each germ layer in the developing mouse embryo from the whole mount level up to the ultrastructural level. Double-labeling on paraffin sections for both this method and TUNEL revealed that cells become Annexin V-biotin labeled early during the process of apoptosis. (C) 1997 Wiley-Liss, Inc.

L11 ANSWER 11 OF 16 MEDLINE on STN DUPLICATE 2  
ACCESSION NUMBER: 97058470 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 8902790  
TITLE: Antibodies against phospholipids other than cardiolipin: potential roles for both phospholipid and protein.  
AUTHOR: Rauch J; Janoff A S  
CORPORATE SOURCE: Montreal General Hospital Research Institute, Quebec, Canada.  
SOURCE: Lupus, (1996 Oct) Vol. 5, No. 5, pp. 498-502. Ref: 62  
Journal code: 9204265. ISSN: 0961-2033.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
General Review; (REVIEW)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199702  
ENTRY DATE: Entered STN: 27 Feb 1997  
Last Updated on STN: 27 Feb 1997  
Entered Medline: 12 Feb 1997

AB Autoantibodies to phospholipids other than cardiolipin have received less

attention, to date, than anti-cardiolipin antibodies. This review focuses on these antibodies and potential roles for both phospholipid and protein in their reactivity. We review data in the literature indicating that antibodies to phosphatidylethanolamine and some lupus anticoagulant antibodies recognize phospholipid-binding proteins in association with phospholipid. Kininogens appear to be involved in the binding of antibodies to phosphatidylethanolamine, while phosphatidylserine-binding proteins, such as prothrombin and annexin V, have been implicated in lupus anticoagulant antibody recognition. These proteins bind to phospholipids that normally reside in the inner monolayer of the cell membrane, suggesting that exposure of these lipids is necessary for protein binding and antibody recognition to occur. In contrast, other autoantibodies, in particular those reactive with erythrocytes, appear to be directed at phospholipids that normally occur in the outer membrane leaflet, such as phosphatidylcholine. In summary, there is clearly accumulating evidence that antibodies to phospholipids other than cardiolipin recognize epitopes on phospholipid-binding proteins. It is not clear whether recognition of these epitopes is due to an increase in antigen density or a change in the protein or phospholipid structure, but it is likely that both protein and phospholipid structure play an important role in the *in vivo* interactions of these antibodies.

L11 ANSWER 12 OF 16 HCPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 1991:79799 HCPLUS  
DOCUMENT NUMBER: 114:79799  
TITLE: Effective induction of anti-phospholipid and anticoagulant antibodies in normal mouse  
AUTHOR(S): Igarashi, Kouji; Umeda, Masato; Tokita, Shigeru; Nam, Kyung Soo; Inoue, Keizo  
CORPORATE SOURCE: Tokyo Res. Cent., Tosoh Corp., Ayase, 252, Japan  
SOURCE: Thrombosis Research (1991), 61(2), 135-48  
CODEN: THBRAA; ISSN: 0049-3848  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Anti-phosphatidylserine (anti-PS) antibodies of the IgG isotype in the serum of BALB/c mouse were effectively induced by the intrasplenic immunization of phosphatidylserine (PS), while only a slight increase of the titer was observed when the antigen was injected i.v. The serum antibodies cross-reacted extensively with other acidic phospholipids, but not with phosphatidylcholine (PC). A remarkable frequency of anti-PS mAbs of the IgG isotype was also observed even when mAbs were produced 10 days after the intrasplenic injection of the antigen. Reactivities of the fifteen mAbs, which had been established from BALB/c mice by the intrasplenic immunization with PS, were further analyzed. Among the fifteen mAbs examined, thirteen cross-reacted with ssDNA and two bound to dsDNA. Seven mAbs had lupus anticoagulant activity and four bound to cultured human umbilical vein endothelial cells (HUVECs). No correlation was found among phospholipid specificities, anti-DNA, anticoagulant, and HUVEC binding activities. One mAb of the IgG2b subclass, named PSG3, which had been produced 10 days after the immunization, was shown to be have a strong lupus anticoagulant, dsDNA binding, and HUVEC binding activities.

L11 ANSWER 13 OF 16 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN

ACCESSION NUMBER: 1989:88137 BIOSIS  
DOCUMENT NUMBER: PREV198936044228; BR36:44228  
TITLE: THE INTERACTION OF LUPUS ANTICOAGULANTS WITH HUMAN PLATELETS.  
AUTHOR(S): MIKHAIL M H [Reprint author]; HEINE M J; PENG O V; SHAPIRO S S  
CORPORATE SOURCE: CARDEZA FOUNDATION HEMATOL RES, JEFFERSON MED COLL, PHILADELPHIA, PA, USA

SOURCE: Circulation, (1988) Vol. 78, No. 4 PART 2, pp. II514.  
Meeting Info.: 61ST SCIENTIFIC SESSIONS OF THE AMERICAN  
HEART ASSOCIATION, WASHINGTON, D.C., USA, NOVEMBER 14-17,  
1988. CIRCULATION.  
CODEN: CIRCAZ. ISSN: 0009-7322.

DOCUMENT TYPE: Conference; (Meeting)

FILE SEGMENT: BR

LANGUAGE: ENGLISH

ENTRY DATE: Entered STN: 28 Jan 1989  
Last Updated on STN: 28 Jan 1989

L11 ANSWER 14 OF 16 HCPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 1986:531837 HCPLUS

DOCUMENT NUMBER: 105:131837

ORIGINAL REFERENCE NO.: 105:21253a,21256a

TITLE: Demonstration of lupus anticoagulant  
antigens using an enzyme-linked immunoadsorbent assay  
(ELISA)

AUTHOR(S): Branch, D. Ware; Rote, Neal S.; Scott, James R.

CORPORATE SOURCE: Sch. Med., Univ. Utah, Salt Lake City, UT, 84132, USA

SOURCE: Annals of the New York Academy of Sciences (1986),  
475(Autoimmun.: Exp. Clin. Aspects), 370-2  
CODEN: ANYAA9; ISSN: 0077-8923

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Using an ELISA, it was found that human lupus anticoagulant  
binds to cardiolipin and phosphatidylserine. Phosphatidylserine is the  
antigen recognized by lupus anticoagulant in coagulation assays  
since cardiolipin is not a component of the thromboplastin used.

L11 ANSWER 15 OF 16 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on  
STN

ACCESSION NUMBER: 1985:333811 BIOSIS

DOCUMENT NUMBER: PREV198580003803; BA80:3803

TITLE: ANTI-PHOSPHOLIPID ANTIBODIES IN SYPHILIS AND A THROMBOTIC  
SUBSET OF SYSTEMIC LUPUS ERYTHEMATOSUS DISTINCT PROFILES OF  
EPITOPE SPECIFICITY.

AUTHOR(S): COLACO C B [Reprint author]; MALE D K

CORPORATE SOURCE: DEP IMMUNOLOGY, MIDDLESEX HOSP MED SCH, ARTHUR STANLEY  
HOUSE, 40-50 TOTTENHAM ST, LONDON W1P 9PG, UK

SOURCE: Clinical and Experimental Immunology, (1985) Vol. 59, No.  
2, pp. 449-456.  
CODEN: CEXIAL. ISSN: 0009-9104.

DOCUMENT TYPE: Article

FILE SEGMENT: BA

LANGUAGE: ENGLISH

AB In a study of connective tissue and infectious disease sera, IgM and IgG  
anti-cardiolipin activity was demonstrated by a solid phase  
radioimmunoassay in systemic lupus erythematosous (SLE), rheumatoid  
arthritis, syphilis and in acute malaria caused by 4 different species of  
Plasmodium [P. falciparum, P. vivax, P. ovale and P. malariae]. The  
highest values were noted in SLE (IgM anti-cardiolipin  $P < 0.005$ , IgG  
anti-cardiolipin  $P < 0.01$ ), but there was no correlation with antibody to  
double-stranded DNA, rheumatoid factor or VDRL [Venereal Disease Research  
Laboratory] titers in any disease group. Anti-cardiolipin binding was  
significantly associated with the lupus anticoagulant,  
thrombocytopenia, spontaneous abortions and thromboses in the SLE patients.  
Ten SLE sera from the thrombotic subset and 10 syphilitic sera with similar  
anti-cardiolipin activity were tested against 4 phospholipid antigens and  
showed significantly different anti-phosphatidylethanolamine/anti-  
phosphatidylserine binding ratios ( $P < 0.001$ ). These  
differences in phospholipid epitope specificity could explain the  
specificity of the VDRL antigen in syphilis serology. A putative role for  
anti-phosphatidylserine in the thrombotic diathesis of SLE is discussed.

L11 ANSWER 16 OF 16 HCPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 1984:588884 HCPLUS

DOCUMENT NUMBER: 101:188884

ORIGINAL REFERENCE NO.: 101:28569a,28572a

TITLE: Behavior of blood proteins at the interface with procoagulant phospholipids and anticoagulant heparin or polymeric biomaterials: a fluorescence study

AUTHOR(S): Dachary, J.; Dulos, E.; Faucon, J. F.; Boisseau, M. R.; Dufourcq, J.

CORPORATE SOURCE: Cent. Rech. Paul Pascal, Domaine Univ., Talence, 33405, Fr.

SOURCE: Colloids and Surfaces (1984), 10, 91-9

CODEN: COSUD3; ISSN: 0166-6622

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Changes in the fluorescence of factors IX and II in the presence of Ca at procoagulant phospholipid interfaces suggest a conformational change of the proteins upon binding. Shifts of transition temperature of phosphatidylcholine-phosphatidylserine mixts. towards those of the pure lecithin component when Ca and coagulation factors are added suggest that lateral phase separation does exist. The higher resonance-energy transfer efficiency with charged pyrene-labeled phospholipids leads to the conclusion that within the membrane, factor II and IX binding sites are domains of phosphatidylserine. Ca-independent phospholipid binding, selective for phosphatidylserine, is well documented and is proposed to be mediated by electrostatic forces between the arginine and lysine residues of the proteins and the neg. charges of the phosphatidylserine. The binding of heparin or the synthetic anticoagulant polystyrene (bearing SO<sub>3</sub><sup>2-</sup> and glutamic acid groups) with antithrombin or thrombin induces different fluorescence changes in these proteins which cause local changes in the tryptophan residues of the proteins upon binding. The interaction of heparin with various mixts. of thrombin and antithrombin indicates that heparin does not modify the structure of the preformed thrombin-antithrombin complex. as far as can be seen by spectrofluorimetry.